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(54) ENZYME TREATMENT OF LEGUMES AND DERIVED PRODUCTS

(71) We, CIBA-GEIGY A.G. a body corporate, organised according to the laws of Switzerland, of 4002 Basle, Switzerland, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

The present invention concerns a process for an enzyme treatment of legumes and their derived products, which can be utilized for human and/or animal nutrition. By the term legumes plants of the family fabaceae such as peas and beans, particularly soybeans are to be understood.

Due to their high protein content legumes are very valuable for human and animal nutrition. In particular, products from soybeans, e.g. soya flakes, soya grits, soybean meal, soymilk and TVP (textured vegetable protein) products provide a valuble protein source. Such products are widely added to food, e.g. bakery products and sausages, for improving their structure and consistency. (J. Agr. Food Chemistry *18* (6) 969 ff (1970).)

The use of the above mentioned products for human nutrition is limited, especially in the case of children, due to the presence of components which cause severe digestive disorders (Med. Klin. *65*, No. 25, 1204—120) (1970) and J. Agr. Food Chem. *18* 977 ff (1970)).

α-Galacto-oligosaccharides are among the factors causing these disorders. (J. Food Science *35*, 634—639 (1970).) Defatted soymeal contains, e.g. approximately 7% of α-galacto-oligosaccharides (approx. 5% Starchyose, approx. 2% Raffinose). These oligosaccarides can be removed from soya products by extraction with water or aqueous alcohol. The resulting soy concentrates do not contain α-galacto-oligosaccharides. The same applies to the so-called Soy isolates which are produced by iso-electric precipitation from alkaline solutions. Soy concentrates and soy isolates are 3 to 5 times more expensive than the above mentioned products, which are produced simply from soybeans by dehulling, grinding, extraction with water, defatting and heat-treatment. Resulting products, e.g. soy flakes, soy grits, soy meal, soymilk and also extruded soy-products (TVP; British Patent Specification No. 1,049,848), however, contain the troublesome α - galacto - oligosaccharides. Due to economic considerations, the incorporation of soy-protein into milk-replacers has been tried repeatedly (O.M. Radostits and J. M. Bell, Can. J. Animal Sci., December 1970, and Z. Nitsan et al., J. Dairy Sci. *54* (1971) 1294). The results of these experiments were poor because the soy was not well accepted by young animals. Four factors are commonly held responsible:

a) Presence of Trypsin inhibitors which interfere with normal digestion of soy-protein.
b) Presence of haemagglutinins.
c) Insufficient proteolytic activity in the gastro-intestinal tract of the young animals.
d) Presence of unresorbable carbohydrates which cause fermentative diarrhoea.

The object of the present invention is a process which allows a simple reduction of these factors.

An attempt to improve the tolerance towards a soy-product by enzymatic degradation of the above mentioned α-galacto-oligosaccharides has been reported. Soymilk was treated at pH 6.2 by a purified α-galactosidase, which had been isolated from MOLSIN (registered Trade Mark), a commercial enzyme preparation produced from Aspergillus satoi ,Seishin Pharmaceutical Co. Ltd., Noda-Chiba, Japan) (J. Food Science, *35*, 655 (1970)).

An improvement in the acceptability of soy for the rearing of calves is claimed for the treatment of soy meal with pectinase or mixtures of pectinase and cellulose (French patent application No. 2,013,753).

These two methods for the enzyme treatment of soya or derived products do not constitute a satisfactory solution. In the first instance, the use of an α-galactosidase from MOLSIN is limited to the treatment of soymilk. In the second case, the amount of soy

which can be incorporated into the milk-replacer has been increased only from 5 to a maximum of 25%.

The present invention consists of a treatment of legumes or derived products in an aqueous suspension or in solution in the pH range 3 to 7, preferably between 4 and 6, with 0.05 to 5% (w/w), preferably 0.1 to 2% (w/w), of an enzyme which enzyme is derived from Aspergillus inuii IAM* 2268 (R-3021) which is produced from this micro-organism by fermenting sterilized wheatbran with a water content of 50% (w/w) at 30°C for 48—72 hours, extracting with water, precipitating with ethanol and drying.

The treatment according to the invention takes place between 10 and 80°C, preferably between 20 and 70°C. The duration of the treatment will be between one minute and 24 hours, depending on the type of bean or derived product and amount of enzyme used.

Soyproducts which can be treated by this process are namely: soy flakes, soy grits, soy meal and soymilk. The treatment of soy flakes, soy grits and soy meal can be carried out with full-fat, partially defatted or totally defatted soy products.

The enzyme treatment can be carried out at any suitable point in the production process, e.g. in the production of soy grits, soy flakes or soy meal the treatment can be applied after grinding the dehulled soybeans. These may or may not be defatted.

The following may be taken as an example describing the invention. Soybean meal is suspended with dilute acetic acid to a pH-value between 4 and 5, in a jacketed container. The required amount of enzyme is added to the suspension, which has previously been heated to 60°C. The reaction mixture is stirred at 60°C for four hours. At the end of the treatment, the mixture is heated to, and maintained at 90°C for 10 minutes. The resulting material is preferably spray-dried. To facilitate this, dilution with water may be required. A second possibility is to dry the material in a rotary vacuum-drier. Soymaterial produced in this way can also be extruded to advantage (TVP-process).

A similar enzyme treatment can also be applied to soymilk.

Products treated in this fashion are tolerated much better by children and young animals than the corresponding untreated products, due to a lower content of α-galacto-oligosaccharides. Furthermore the treatment results in a partial digestion of high molecular weight-proteins giving low molecular weight-proteins, peptides, and amino acids. This imparts a better digestibility of these materials for children and young animals.

The following examples will serve to illustrate preferred methods of enzymatic treatment of legumes. It is understood that these examples are set forth merely for illustrative purposes.

*IAM = Institute of Applied Microbiology, University of Tokyo.

Example 1

Treatment of Soy meal for human nutrition

Nutrisoy 220 (Archer Daniels Midland Co., U.S.A.):

Protein content	39 % (w/w) (Kjeldahl-N × 5.7)
Fat content	24 % (w/w)
Stachyose	3.8% (w/w)
Raffinose	0.8% (w/w)

Enzyme from Aspergillus inuii IAM 2268 (R-3021):

The enzyme is produced from this micro-organism by fermenting sterilized wheatbran with a water content of 50% (w/w), at 30°C for 48—72 hours, extraction with water, precipitation with ethanol and drying.

50 grams Nutrisoy 220 was suspended in 200 ml of diluted acetic acid (0.5%) resulting in a pH-value of 4.8. 0.5 gram of enzyme was added with stirring to the suspension which had been warmed to 55°C. The enzyme treatment was terminated by heating to 90°C. Spray-drying or vacuum-drying served to remove the water and acetic acid. A quantitative yield of treated soy meal was obtained. Galactose analysis of an aqueous extract of this product showed that more than 50% of the initial galacto-oligosaccharides had been degraded. In a parallel experiment 0.5 grams of MOSLIN (Seishin Pharmac. Co., Noda-Chiba, Japan) was used instead of the enzyme from aspergillus inuii. Determination of liberated galactose showed less than 6% degradation of galacto-oligosaccharides. In a control experiment, without enzyme, no galactose could be detected.

Example 2

50 grams of Nutrisoy 220 was treated with 0.1 gram of the enzyme from Aspergillus inuii at a pH-value of 4.8 as in Example 1. The mixture was stirred at 60°C for 4 hours. The degradation of galacto-oligosaccharides was more than 50%. In a parallel experiment, utilizing 0.1 gram MOLSIN the degradation of galacto-oligosaccharides measured as liberated galactose was less than 4%.

Example 3

Treatment of Soybean meal for animal nutrition

Defatted toasted soybean meal

Protein content	44% (w/w) (Kjeldahl-N × 5.7)
Fat content	2% (w/w)

50 grams of soybean meal was suspended in

250 ml of diluted acetic acid (0.5%) resulting in a pH-value of 4.9. The suspension was heated to 38°C and 0.8 grams of the enzyme from Aspergillus inuii was added. The suspension was stirred from 8 hours at 38°C. Further treatment as in Example 1.

The degradation of galacto-oligosaccharides measured as liberated galactose was more than 60%.

Example 4

100 kg of finely milled soybean meal was suspended in a mixture of 300 l of water and 2 l of concentrated acetic acid in a jacketed, stirred vessel. This resulted in a pH-value of 4.8.

0.6 kg of enzyme from Aspergillus inuii was added with stirring. This mixture was held between 50 and 55°C for 16 hours. The contents of the vessel were then rapidly cooled to room-temperature.

10 N Sodium hydroxide was added to produce a pH-value of 6.5. A buff coloured powder was obtained by spray drying this aqueous suspension. The spray-drying was conducted using a Twin-fluid atomizer (manufactured by Industriewerke Karlsruhe) with an aperture of 3 mm. acting at 3 atms. air pressure, an air entry temperature between 180 and 185°C and an outlet temperature between 90 and 95°C.

	Enzyme-treated Soybean meal	Untreated Soybean meal
water content	5 %	10 %
Nitrogen content	7.3%	7.3%
"Nitrogen Solubility Index" (NSI)*	70	15
total reducing sugar (measured by Somogyi-Nelson reagent) expressed in mg equivalent glucose/g soya	140	2
α-galactoside**	12	36

* NSI: Smith et al., Cereal Chem. *43*(1966), 261

** Releasable galactose determined photometrically by galactose-dehydrogenase and Di-phosphopyridinenucleotide (expressed in mg galactose/gram soya).

Microbial counts (bacteria and fungi) are less in the enzyme-treated soybean meal than in the untreated soybean meal. The enzyme-treated soybean meal disperses readily in warm water and is compatible in all proportions with powdered dried skimmed milk. The resulting milk-like suspension is readily accepted by young calves.

Example 5

Enzyme treatment of "Navy Beans" (Phaseolus vulgaris)

3 kg of these beans were suspended in 9 l of diluted acetic acid resulting in a pH-value 4.8. 30 grams of enzyme from Aspergillus inuii were added and the mixture stirred for 20 hours at 50°C.

Freeze-drying resulted in a bean-powder whose residual content of α-galacto-oligosaccharides was 18% of that contained in the original beans.

Example 6

Enzyme treatment of "California Small White Beans"

The enzyme treatment was carried out as in Example 5. Freeze-drying resulted in a bean-powder in which no residual content of α-galacto-oligosaccharides could be detected.

WHAT WE CLAIM IS:—

1. A method of improving the acceptability of a legume or its derived products (as herein defined), which method comprises treating an aqueous suspension or solution containing the legume or its derived products at a pH from 3 to 7 with 0.05% to 5% (w/w) of an enzyme which enzyme is derived from *Aspergillus inuii* IAM 2268 (R-3021) by fermenting sterilised wheatbran with a water-content of 50% (w/w) at 30°C for a period from 48 to 72 hours; extracting with water; precipitating with ethanol; and drying.

2. A method according to claim 1 wherein soy beans or their derived products are treated.

3. A method according to claim 1 or 2 wherein the treatment takes place at a pH from 4 to 6.

4. A method according to any preceding claim wherein from 0.1% to 2% (w/w) enzyme to legume or derived product is used.

5. A method according to any preceding claim wherein the treatment takes place at a temperature from 20°C to 70°C.

6. A method according to any preceding claim substantially as hereinbefore described with reference to the foregoing Examples.

7. A legume or its derived product whenever improved by a method according to any preceding claim.

8. A human foodstuff or animal feedstuff comprising a legume or its derived product according to claim 7.

Agents for the Applicants
GALLAFENT & CO.,
Chartered Patent Agents,
8 Staple Inn,
London, WC1V 7QH.

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